

**We Claim:**

1. A method of screening a test nucleic acid sequence to identify a candidate nucleic acid sequence encoding an antimicrobial peptide, said method comprising:
  - 5 (a) identifying an initial peptide of interest;
  - (b) identifying a genomic DNA sequence from a first fish species containing a nucleotide sequence encoding the initial peptide;
  - (c) identifying within the genomic DNA sequence a flanking nucleotide sequence on each side of the peptide-encoding sequence;
  - 10 (d) obtaining a primer oligonucleotide sequence complementary to each flanking sequence; and
  - (e) screening a test nucleic acid sequence from a fish species other than the first fish species to determine whether it is capable of being amplified by PCR using the primers from step (d);
  - 15 amplification indicating that the test nucleic acid sequence is a candidate nucleic acid sequence encoding an antimicrobial peptide.
2. The method of claim 1 wherein the initial peptide has a net positive charge of at least 2 and has an amphipathic structure.
3. The method of claim 1 wherein the initial peptide is selected from the group consisting of a hepcidin, a pleurocidin, a pardaxin, a misgurin, HFA-1, a piscidin, a moronecidin, a parasin, and a cleavage product of histone 2A from catfish other than a parasin.
4. The method of any one of claims 1 to 3 comprising a further step (f) of predicting the amino acid sequence encoded by the candidate sequence and  
25 selecting nucleic acid sequences which are predicted to encode peptides having an amphipathic structure and a net charge.

5. The method of claim 4 comprising a further additional step of obtaining a peptide corresponding to the candidate nucleic acid sequence and assaying the peptide sequence for antimicrobial activity.
6. The method of claim 1 comprising a further step (a') of confirming that  
5 the initial peptide has antimicrobial activity.
7. The method of claim 1 wherein the initial peptide is a pleurocidin.
8. The method of claim 7 wherein at least one of the flanking sequences is selected from the group consisting of a nucleotide sequence encoding signal sequence I, a nucleotide sequence encoding Acidic Sequence I,  
10 GCCCACTTTGTATTCGCAAG and CTGAAGGCTCCTTCAAGGCG.
9. The method of claim 1 wherein the initial peptide is a hepcidin.
10. The method of claim 9 wherein at least one flanking sequence is selected from the group consisting of a nucleotide sequence encoding signal peptide II, a nucleotide sequence encoding signal peptide III, a nucleotide  
15 sequence encoding signal peptide IV, a nucleotide sequence encoding signal peptide V, a nucleotide sequence encoding prosequence I, a nucleotide sequence encoding prosequence II, ACAACCTCGTCCTTAGG and ACGCCCGTCCAGGAAT.
11. An isolated nucleic acid sequence identifiable using the method of any  
20 preceding claim.
12. An isolated polypeptide capable of being encoded by the nucleic acid sequence of claim 8.
13. An isolated nucleic acid sequence comprising a flanking sequence.
14. A kit comprising:

- 5
- a. a first nucleic acid sequence at least 95 % identical to a first flanking sequence, located at or near a 5' end of a target sequence encoding an antimicrobial peptide;
  - b. a second nucleic acid sequence at least 95 % identical to a second flanking sequence located at or near a 3' end of a target sequence encoding an antimicrobial peptide; and
  - c. instructions for carrying out the method of claim 1.

10 15. Use of at least one of signal sequence I, acidic sequence I, signal peptide II, signal peptide III, signal peptide IV, signal peptide V, prosequence I, prosequence II, nucleic acid sequences encoding them, and nucleic acid sequences substantially complementary to such encoding nucleic acids, in the method of claim 1.

16. An isolated antimicrobial peptide at least 80% homologous to one of peptide a, b, c or d:

- 15
- |                     |                         |
|---------------------|-------------------------|
| <u>Peptide a</u>    | GW(G/K)XXFXK            |
| <u>Peptide b</u>    | GXXXXXXXXHXGXXIH        |
| <u>Peptide c</u>    | FKCKFCCGCCXXGVCGXCC     |
| <u>Peptide d</u>    | CXXCCNCC(K/H)XKGCGFCCKF |
| <u>Peptide e</u>    | FKCKFCCGCRGXXCGLCCKF    |
| 20 <u>Peptide f</u> | XXXCXXCCNXXGCGXCCKX     |

17. The antimicrobial peptide of claim 13 which is at least 90% homologous to one of peptide a, b c or d.

18. The antimicrobial peptide of claim 13 which is one of peptide a, b, c or d.

19. An isolated nucleic acid sequence depicted in Appendix I or Appendix II.

20. A method of screening a test nucleic acid sequence to identify a candidate nucleic acid sequence encoding an antimicrobial peptide, said peptide comprising:

- a) identifying a nucleic acid sequence encoding an initial peptide of interest;
- 5 (b) identifying a genomic DNA sequence from a first fish species containing a nucleotide sequence encoding the initial peptide;
- (c) identifying within the genomic DNA sequence a flanking nucleotide sequence on each side of the peptide-encoding sequence;
- (d) obtaining a primer oligonucleotide sequence complementary to each  
10 flanking sequence; and
- (e) screening a test nucleic acid sequence from a fish species other than the first fish species to determine whether it is capable of being amplified by PCR using the primers from step (d);

15 21. An isolated peptide selected from the group consisting of:

- (a) WLRRIGKGVKIIIGGAALDHL;
- (b) GRRKRKWLRRIGKGVKIIIGGAALDHL;
- (c) RWGKWFKKATHVGKHVGKAALTAYL;
- (d) RSTEDIISISGGGFLNAMNA;
- 20 (e) FFRLLFHGVHHGGGYLNAA;
- (f) FFRLLFHGVHHVGKIKPRA;
- (g) GWKSVFRKAKKVGKTVGGLALDHYL;
- (h) GWKKWFNRAKKVGKTVGGLAVDHYL;
- (i) GWRTLLKKAEVKTVGKLALKHYL;
- 25 (j) AGWGSIFKHIFKAGKFIHGAIAHND;
- (k) GFWGKLFLGLHGIGLLHLHL;
- (l) GWKKWLRKGAKHLGQAAIK;
- (m) GWKKWLRKGAKHLGQAAIKGLAS;
- (n) GWKKWFTKGERLSQRHFA;

- (o) FLGLLFHGVHHVGKWIHGLIHGHH;  
(p) GFLGILFHGVHHGRKKALHMNSERRS;  
(q) FLGFLFHGIHHGIRAIHLIHG;  
(r) FFGALIKGAIHGGKLLHKLKKHEHHGYGKHWG;  
5 (s) FLGFLFHGIRHGIKAIHGMIHG;  
(t) GKGRWLERIGKAGGIIIGGALDHLG;  
(u) GLGNWMGPHISGEKKALHMNSERRS;  
(v) GLGNWIVRPIGGEKKALQMNSERRS;  
(w) LFGKFLKKVVHAGTSIGETALHVAAEHHGLHAHHG;  
10 (x) GLGNWMGPHISGRKKALHMNSERRS;  
(y) FLGLLFHGVHHVGKLIHGLIHG;  
(z) ARWGTFFKHIFKAGRFIHGAIQAHNDG;  
(aa) AWIPALNRIYHGALLRINRQMVMYYRRHWHG;  
(ab) AWMPALNRIYHGALLRINRQMVMYYRRHWHG;  
15 (ac) GWKKWFTKGAKHLGQAAINGLAS;  
(ad) GWKKWLRKGAKHLGQAAIKGLAS;  
(ae) FGDFYMKPGRKISHGYIRSPYG;  
(af) GYWRFRNHRGERLSQRHFA;  
(ag) FGMLFHRVHHAGRLIHRFIKRHG;  
20 (ah) IFGLIATAVHNAGRLIHRLLGFHHGPPGFWHG;  
(ai) IFGLIATAVHNVGRLVHGLLGFHHGPPGFWHG;  
(aj) IFGLIATAVHNVGRLVHGLLGFHHGPPRFWHG;  
(ak) FFGMRFHGVHHAGGGFLNAQGLLPSLLLNPgyRG;  
(al) FFGALLKGAQALHGIIHNARHG;  
25 (am) GWKDWFRKAKKVGKTVGGLALNHYLg;  
(an) GIRKWFKKAAHVGKEVGKVALNACL;  
(ao) GLKKWFKKAVHVGKKVGKVALNAYLG;  
(ap) GWRKWIKKATHVGKHIGKAALDAYIG;  
(aq) GCKKWFKKAAHVGKNVGKVALNAYLG;  
30 (ar) GIRKWFKKAAHVGKKVGKVALNAYLG;  
(as) WLERKWFKKATHVGKHVGKAALDAYLG;  
(at) FFGLLFHGIHHAGKLIHGLIHG;

- (au) LGNWMGPHISGRKKALQMNSERRS;  
 (av) FLGLLFHGVHHVGNLIHGLIHHG;  
 (aw) GIRKWFKKAHVGGKVGKVALNAYLG;  
 (ax) a C-terminally amidated or otherwise C-terminally or N-terminally modified peptide of (a) to (z) or (aa) to (aw);  
 (ay) a C-terminally amidated peptide of (a) to (z) or (aa) to (aw) where modification replaces C-terminal G; and  
 (az) a peptide of (a) to (z) or (aa) to (aw) comprising at least one conservative amino acid substitution or deletion of an amino acid residue thereof.

22. An isolated nucleotide sequence encoding a peptide of claim 21.

23. An isolated peptide selected from the group consisting of:

- (a) MKTFSVAVAVVVVLACMFILESTAVPFSEVRTEEVE SIDSPVGEHQQ-PGGTSMNLPMHFRFRKRQSHLSLCRWCCNCCHNKGCGFCCKF;  
 (b) MKTFSVAVAVVVVLACMFILESTAVPFSEVRTEEVE SIDSPVGEHQ-QPGGTSMNLPMHFRFRKRQSHLSLCRWCCNCCHNKGCGFCCKF;  
 (c) MKAFSVAVVLVIACMFILESTAVPFSEVRTEEVEG SFDSPVGEHQQP-GGESMHLPEPFRFRKRQIHLSLCGLCCNCCHNIGCGFCCKF;  
 (d) RTEEVE SIDSPVGEHQQP GGTS MNLPMHFRFRKRQSHLSLCRWCCNCCHNKGCGFCCKF;  
 (e) MKTFSVAVVPVIACMFILESTAVPFSEVRTEEVEG SFDSPVGEHQQP-GGTSMNLPMHFRFRKRQSHLSLCRWCFNCCHNKGCGFCCKF;  
 (f) MKQFSVAVVLVMACMFIVESTAVPFSEVRTEEVEGSLDSPVGEHQQ-PGGESMHLPEPFRFRKRQIHLSLCGLCCNCCHNIGCGFCCKF;  
 (g) MKAFSIAVAVTLVLAFCIQCSSAVPFQGVQELEEAGGNDTPVAEH-QVMSMESWMENPTRQKRHISHISLCRWCCNCCKANKGCGFCCKF;  
 (h) MKTFSVAVAVTLVLAFCIQDSSAVPFQGVQELEEAGGNDTPVAAH-QMMSMESWMESPVRQKRHISHISMCRWCCNCCKAKGCGPCCKF;  
 (i) MKTFSVAVTVAVVLVFICIQSSGTFPEVQELEEAVSNDNAAA EHQ-ETSVDSWMMMPYNRQKRAFKCKFCCGCCRAGVCGLCCKF;

- (j) MKTFSVAVTVAVVLVFICIQSSASFPEAQELEEEAVSNDNAAAEHQ-  
ETPVDSWMPYNRQKRSFKCKFCCGCCRAGVCGLCCKF;
- (k) MKTFSVAVTVAVVLVFICIQSSASFPEAQELEEEAVSNDNAAAEHQ-  
ETPVDSWMPNNRQKRGFKCKFCCGCCRAGVCGLCCKF;
- 5 (l) MKTFSVAVTVAVVLVFICIQSSATFPEMPYNRQKRGFKCKFCCG-  
CCGAGVCGMCCKF;
- (m) MKTFSVAVTVAVVLVFICIQSSASFPEAQELEEEAVSNDNAAAEHQ-  
ETPVDSRIPYNRQKRSFKCKFCCGCCRAGVCGLCCKF;
- (n) MKTCSVAVTVAVVLVFICIQSSASFPEVQELEEAVSNDNAAAEHQ-  
10 ETPVDSWMPNNRQKRGFKCKFCCGCCRAGVCGLCCKF;
- (o) MKTISVAVTVAVVLVFICIQSSASFPEAQELEEEAVSNDNAAAEHQE-  
TPVDSGMIPYNRQKRSFKCKFCCGCCRAGVCGLCCKF;
- (p) MKTFSGAVTVAVVLVFICIQSSASFPEVQELEEAVSNDNAAAEHQ-  
ETPVDSWMPNNRQKRGFKCKFCCGCCRAGVCGLCCKF;
- 15 (q) MKTSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAAHQ-  
ETSVDSWMPYNRPKRSFKCKFCCGCCRA-GVCGLCCKF;
- (r) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAEHQ-  
ETSVDSWMPYNRPKRSFKCKFCCGCCRAGVCGLCCKF;
- (s) MKTFVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAEHQ-  
20 ETSVDSWMPYNRQKRSFKCKFCCGCCRAGVCGLCCKF;
- (t) MKTSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAAHQ-  
ETSVDSWMPYNRQKRSFKCKFCCGCCRAGVCGLCCKF;
- (u) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAEHQ-  
ETSVDLWMPYNRQKRGFKCKFCCGCCSPGVCGLCRRF;
- 25 (v) MKTFSVAVAVAVVLIFICIQSSATFPEVQELEEAVSNDNAAAEHQE-  
TSLDSWMPYNRQKRGFKCKFCCGCCRAGVCGLCCKF;
- (w) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAEHQ-  
ETSLDSWMPYNRHKRSFKCKFCCGCCRAGVCGLCCKF;
- (x) MKTFSVAVTVAVVLVFICIQSSATFPEVQELGEAVSNDNAAAEHQ-  
30 ETSVDSWMPYNRPKRSFKCKFCCGCCRAGVCGLCCKF;
- (y) MKTFSVAVTVAVVLIFICIQSSATSPEVQGLEEAVSNDNAAAEHQ-  
ETSVDSWMPYNRQKRGFKCKFCCGCCRPGVCGLCCRS;

- (z) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQ-  
ETSVDLWMMPYNRQKRGFKCKFCCGCCRPGVCGLCCRF;
- (aa) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEH-  
QETSVDL-WMMPYNRQKRGFKCKFCCGCCSPGVCGLCCRF;
- 5 (ab) KTFSSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQET-  
SVDS-WMMPYNRQKRGFKCKFCCGCCSPGVCGLCCCKF;
- (ac) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEH-  
QETSVDS-WMMPYNRQKRGFKCKFCCGCCRPGVCGLCCCKF;
- (ad) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQ-  
10 ETSVDSWMMPYNRQKRGFKCKFCCGCCRPGVCGLCCCKF;
- (ae) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQ-  
ETSVDSWMMPYNRQKRGFKCKFCCGCCRPGVCGLCCRF;
- (af) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSSDNAAAHEHQ-  
ETSVDSWMMPYNRQKRSFKCKFCCGCCRRGVCGLCCCKF;
- 15 (ag) MKTISVAVTVAVVLLFICTQQSSATFPEVQELEEAVSSDNAAAHEHQ-  
ETSVDSWMMPYNRQKRGFKCKFCCGCRCGALCGLCCCKF;
- (ah) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEPVSSDNAAAHEH-  
QETSVDSWMMPYNRQKRGFKCKFCCGCRCGALCGLCCCKF;
- (ai) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSSDNAAAHEHQ-  
20 ETSVDSWMMPYNRQKRGFKCKFCCGCRCGALCGLCCCKF;
- (aj) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQ-  
ETPVDSGMMPNNRQKRSADCWPCCNQNCGTCCKV;
- (ak) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEH-  
QETSVDSWMMPYNRQKRSAECSFCCNESGCGICCKF;
- 25 (al) MKTFSVAVTVAVVLVFICIQSSATFPEVQELEEAVSNDNAAAHEHQ-  
ETSVDSWMMPYNRQKRSAECSFCCNESGCGICCKF;
- (am) MPNNRQKRGSNCKPCCNHNGCGTCCEV;
- (an) a C-terminally amidated peptide (a) to (z) or (aa) to (am); and
- (ao) a peptide of (a) to (z) or (aa) to (am) comprising at least one  
30 conservative amino acid substitution of an amino acid residue  
thereof.



24. An isolated nucleotide sequence encoding a peptide of claim. 23.